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Total Agent per Liter of Air With Particle Size Distribution (TALA*p*): A New Unit of Measure for the Test and Evaluation of Biodetectors

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*A key component in the test and evaluation of bioaerosol detectors is the unit of measure used to describe the distributed bioaerosol. We examine two existing units of measure, Agent Containing Particles per Liter of Air (ACPLA), and Biologically Active Units per Liter of Air (BAULA). We conclude that ACPLA is an insufficient unit of measure that provides little useful information about the bioaerosol. While BAULA corrects many of the issues present in ACPLA, calculation of BAULA is extremely difficult as it requires knowledge of a number of variables that are currently unavailable. Therefore, we propose a new unit of measure, Total Agent per Liter of Air with particle size distribution (TALA*p*). TALA*p* contains the two most important variables for evaluating biodetectors: the amount of agent present and the particle size distribution. Thus, TALA*p* allows for more accurate and reproducible testing of biodetectors. Furthermore, TALA*p* can be implemented in sealed test chambers using existing referee equipment and is directly comparable to legacy ACPLA data. While some testing procedures may need to be augmented to measure TALA*p* in breeze tunnel and field testing, these new procedures are relatively simple to implement and will pay additional dividends to the test and evaluation community.*[1]

Key words: Biodetectors; biological agents; health hazards; particle size; TALA*p*; units of measure.

Detection of biological agents is a complex endeavor that represents the intersection of the particular detection technology (e.g., Polymerase Chain Reaction [PCR], light scatter, immunoassays), the nature of the biological agent (e.g., viral, bacterial, toxin), and the ambient environmental conditions. Testing and evaluation (T&E) of prototype biodetectors is therefore complicated by the need to consider these variables and to carefully control testing conditions. These considerations can be further complicated depending on the question of application of the data; that is, whether one is conducting a straightforward T&E assessment of competing biodetection technologies in order to determine their relative capabilities, or using the data to assess health effects and guide subsequent operational decisions. Ideally, a standard unit of measure for a bioaerosol challenge should be applicable to both point and standoff detectors and should be able to be translated into operational decisions.

Agent containing particles per liter of air: an imperfect unit of measure

One historical unit of measure is Agent Containing Particles per Liter of Air (ACPLA). ACPLA can be easily measured for such biological agents as bacterial spores, which are robust enough to survive environmental and collection conditions, and can be cultured using standard laboratory methods. One can calculate

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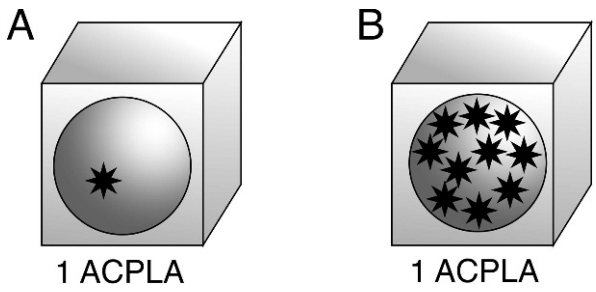

Figure 1. Bioaerosols with the same Agent Containing Particles per Liter of Air (ACPLA) value can be vastly different. Both aerosol particles depicted above share an ACPLA value of 1; there is one agent-containing particle in the surrounding liter of air. However, the threat posed by the aerosol on the right (B) is substantially greater, as it contains 10 times as many biological agents as the aerosol on the left (A). ACPLA contains no measurement of the total number of agents present and, hence, provides very little information about the aerosols being detected.

the number of particles that contain agent (i.e., organism or toxin), but it is impossible to know how much agent is present since one particle could contain one or thousands of agents. For this reason, two aerosols that both have an ACPLA measure of 1 may pose vastly different threats *(Figure 1)*.

The ambiguity in a unit of measure such as ACPLA clearly poses a problem for operational decision making since it provides very little useful information about the total amount of agent present in the environment. From a T&E perspective, ACPLA provides a false sense of accuracy and prevents accurate comparisons between detector technologies. For example, suppose there are two competing technologies, Alpha, which can detect as few as 10 units of agent, and Beta, which can detect a single unit. In this theoretical example, it is clear that Beta has 10 times the sensitivity of Alpha. However, using a unit of measure such as ACPLA, it is absolutely possible that Alpha and Beta could test identically, or that Alpha could actually test as the *better* technology. Using Aerosol B in *Figure 1*, both Alpha and Beta would register as capable of detecting 1 ACPLA and test with equivalent sensitivity. It is important to note that this inaccuracy is not just a theoretical concern; these types of inaccuracies do occur to some extent during actual detector testing.

Problems with ACPLA are further complicated by the fact that the size of the "agent containing particles" is not captured by the unit of measure. A bioaerosol with particles the size of peas poses a relatively low threat since the particles will settle to the ground quickly and can not be readily inhaled. While this example is obviously extreme, particle size is important since particles beyond 10 microns in diameter tend to settle rather quickly and are not efficiently retained in the respiratory tract. This problem is additionally complicated because even in the <10 micron range particle size has a potential effect on infectivity and presentation of the disease by determining where in the airway—sinuses, throat or deep bronchi, for example—the agent is likely to be deposited. Furthermore, many standoff detection systems are very sensitive to particle size. Even if the total number of particles is the same, a detector will react differently if those particles are 1 micron or 5 microns in diameter. Thus, if a Particle Size Distribution (PSD) is not specified within the unit of measure, standoff detector technologies cannot be properly assessed, compared, or evaluated. In summary, ACPLA contains very little useful information that describes the bioaerosol, no information about the total amount of agent or whether it is alive or dead, and can be very difficult to measure for agents other than bacterial spores.

Biologically active units per liter of air: an informative but currently unworkable unit of measure

To address the flaws associated with ACPLA, a new unit of measure, Biologically Active Units per Liter of Air (BAULA), was recommended by a recent National Research Council (NRC) study (NRC 2008). This study also recommended that an additional unit, D*ae*, representing the aerodynamic size of bioaerosol particles, be added to the calculation. In theory, BAULA and D*ae* together provide a single unit of measure that is normalized to health effects and is thus a useful unit with which to make operational decisions. For example, an arbitrary score of "10" for tularemia would indicate the same health hazard as a score of "10" for anthrax even though the number of pathogens (by orders of magnitude) and the PSD differed. Such a metric would address the deficiencies of ACPLA by taking into account the amount of biologically active agent, the agent's PSD, and the infectivity of any given biological agent.

In practice, however, BAULA is virtually impossible to calculate for several reasons. First, the specific health hazard of most List A biological agents is not known, nor is it likely to be known without enormous investments in developing new animal models for these diseases. Second, there is no legitimate way to calculate the viability of the biological agent once it has been distributed as an aerosol. Most agents other than anthrax spores are very labile in the environment and prone to inactivation as a result of temperature, humidity, ultraviolet radiation, dissemination technique, collection technique, and other environmental factors. Moreover, biodetectors based on immunological, nucleic acid (i.e., PCR), or light scattering

detection cannot discriminate active from inactive agent and would thus be incapable of outputting a measurement in BAULA. Third, the effects of PSD, represented by D*ae*, on disposition of biological agents within the human target cannot be reliably estimated. Indeed, calculating health effects based on different particle sizes, as BAULA requires, is arguably impossible as sites of deposition, immune response, and breathing rates will vary greatly between human and animal models. As difficulties in the pharmaceutical industry indicate, tests on animal models may not be indicative of the results found in humans. While animal models are likely to provide the best achievable estimates of health effects, the additional uncertainty in translating those effects to humans provides further justification for leaving health hazard out of a standard unit of measure. The standard unit of measure could then be translated into best estimates of health effects as the situation requires. In addition, particle size calculations are readily achievable in a pristine laboratory environment but are extremely difficult in the field, where background particulates can make up the majority of the sample. In summary, BAULA is a theoretically sound, but currently incalculable, unit of measure. As bioaerosol detectors/referee equipment improve and reliable health hazard information is collected, BAULA may become a more feasible unit of measure and could be considered a "long-term goal" for the biodetector community.

Rationale for a new unit of measure, total agent per liter of air with particle size distribution

With ACPLA insufficient and BAULA/D*ae* impractical, a new unit of measure is clearly needed for T&E protocols. From a T&E perspective, viability of the biological agent in an aerosol is not a concern because the systems being tested detect only presence of an agent and cannot discriminate dead from live agent. From the operational perspective, viability of a biological agent could be useful information; however, given that detection systems cannot determine viability, all commanders will put their troops into a protective posture if any biological agent, living or dead, is detected. The new measure should therefore not take into account the viability of the biological agent. It is important to note that some measure of viability may be required in the *referee equipment* in order to connect test trials to operational needs and capabilities. Unlike detector technologies, referee equipment can take substantial amounts of time to determine viability, opening the door to a host of viability techniques that are not feasible on a detector platform. However, the most important factor in a unit of measure for T&E of detectors is an accurate representation of the total amount of the biological agent present, followed by information on PSD. From a practical and economic point of view, the new unit of measure should be able to be derived with current technology used in test chambers, ambient breeze tunnels, and field testing.

Key components of a unit of measure for bioaerosol testing and evaluation

- Live versus dead agent is irrelevant since most detector technologies cannot discriminate between the two states, and Commanders will decide to implement protection assuming viability is possible.
- The total number of agents present in a given volume of air is the most important measure of a bioaerosol.
- The PSD of a bioaerosol can have a substantial impact on testing results and must be included in a unit of measure.
- The unit must be "measurable" in test chambers, ambient breeze tunnels, and in the field, using readily available technologies.

We here propose a new unit of measure that combines Total Agent per Liter of Air (TALA) with PSD, to be represented as TALA*p*. For example, for an aerosol with 100 organisms per liter of air in a normal PSD centered at 5 microns with a 2-micron standard deviation, the aerosol could be reported as 100 TALA (normal, 5, 2). If a more concise reporting mechanism is required, the volume-weighted average particle radius could be used to describe the PSD instead, since this single value describes the particle size that will contain the largest amount of agent. In this case, TALA*p* would be reported as 100 TALA (5 microns). The logic for this unit of measure is straightforward. TALA*p* contains the most important measure of a bioaerosol, the total amount of agent present, and describes the dissemination of that aerosol via the PSD. For T&E purposes, TALA*p* can be calculated as long as one knows two things: (a) the composition of what is being disseminated (e.g., the amount of agent, fluidizer, media), and (b) the dynamics of the distribution including PSD, flow rate through the detector, and collection time. Crucially, these factors are within the control of the tester.

The first requirement is to know how much agent (using PCR, immunoassays, cell sorters, culture techniques, etc.) and how much inert material (e.g., fluidizer, media) are in the sample that will be disseminated. Given a well-designed sample preparation protocol, this information is readily available. The

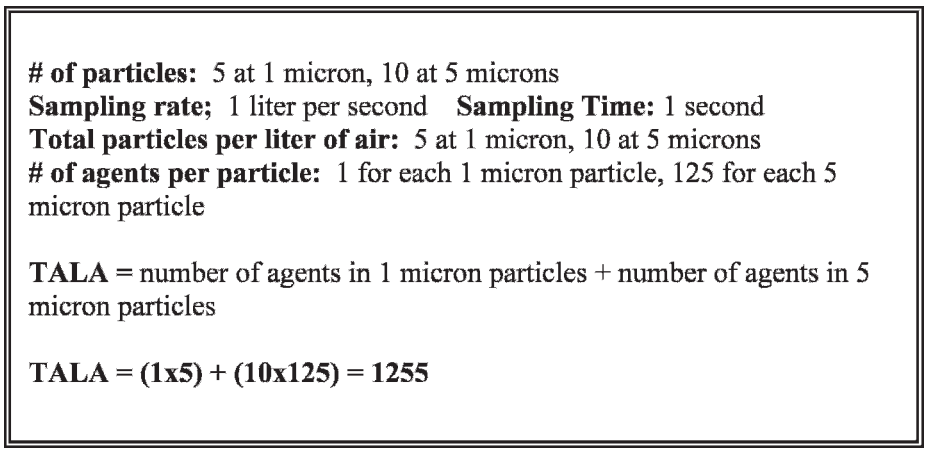

Figure 2. A simplified conceptualization of the calculation of Total Agent per Liter of Air (TALA). The number of particles of differing sizes (1 or 5 microns in this example) can be determined either through direct measurement using a particle sizer or from knowledge of the dissemination technique. From this value, the number of particles per liter of air can be computed using the sampling rate of the particle sizer (chosen as 1 liter per second for simplicity). Knowing the composition of these particles (from the sample preparation protocol or experimental measurement), one can determine the number of agents per particle. By combining these values, the TALA can be calculated.

second requirement is to know the PSD. For a solid powder, the PSD is largely dependent on the degree to which the sample has been milled and is readily available. From this point, the dissemination technique may have little effect on the PSD, though some facilities have indicated an effect can occur. In this case, direct measurement of the resulting PSD via a particle sizer (i.e., TSI Aerodynamic Particle Sizer) may be required. For liquid slurries, the calculation is more complex. Most liquid dissemination techniques are well characterized and generate a fairly consistent PSD. However, since the liquid component of a slurry will rapidly evaporate upon dissemination (leaving only solid components behind), the resulting PSD that is actually presented to a detector will be different. The PSD of this resulting aerosol can still be readily predicted or, in a well-designed test chamber, measured directly assuming the composition of the starting material is well characterized. In fact, some T&E facilities have already created models that can make these particle size predictions. From an experimental point of view, it is preferable to measure PSDs directly via a particle sizer, as this method will account for any agglomeration or de-agglomeration of particles after dissemination.

From here it is relatively easy to conceptualize how TALA would be calculated. Knowing the number of particles being distributed (preferably from direct measurement), the size of these particles (again, preferably from direct measurement), and their composition (from the sample preparation protocol), one can calculate the TALA (see *Figure 2* for a simplified example).

For the test paradigm to be relevant, the PSD must be controlled and applicable to real life biological warfare scenarios. For the purpose of testing, the PSD could be standardized within the respirable range, generally 1–10 microns. Ideally, the PSD will match what is most likely to be experienced during a biological attack. Lacking this important piece of information, detectors could be tested at a variety of PSDs (i.e., tests could be conducted with 1- to 5-micron particles and separately with 5- to 10-micron particles). Beyond stating the range of the PSD, additional information should be recorded about the "shape" of the distribution. Again, the PSD "shape" stated in T&E requirements will ideally match that likely to be seen in a biological attack. Assuming this information is not forthcoming from the intelligence community, a reasonable assumption can be made that most aerosols will not be monodisperse particulates. As such, some arbitrary distribution shape may be necessary (i.e., a normal distribution centered at x with a standard deviation of y). Multiple, well-specified PSDs will be extremely important for testing standoff detectors, which may have vastly different responses depending on the size of the particles *(Figure 3)*.

One can readily see that, within the limits of experimental error, the data required to compute the value for TALAp are available using equipment and procedures already in place in a sealed test chamber. More important, in many cases, one could go back into archival databases and perform these calculations on data from previous tests. Notably, all of the informa-

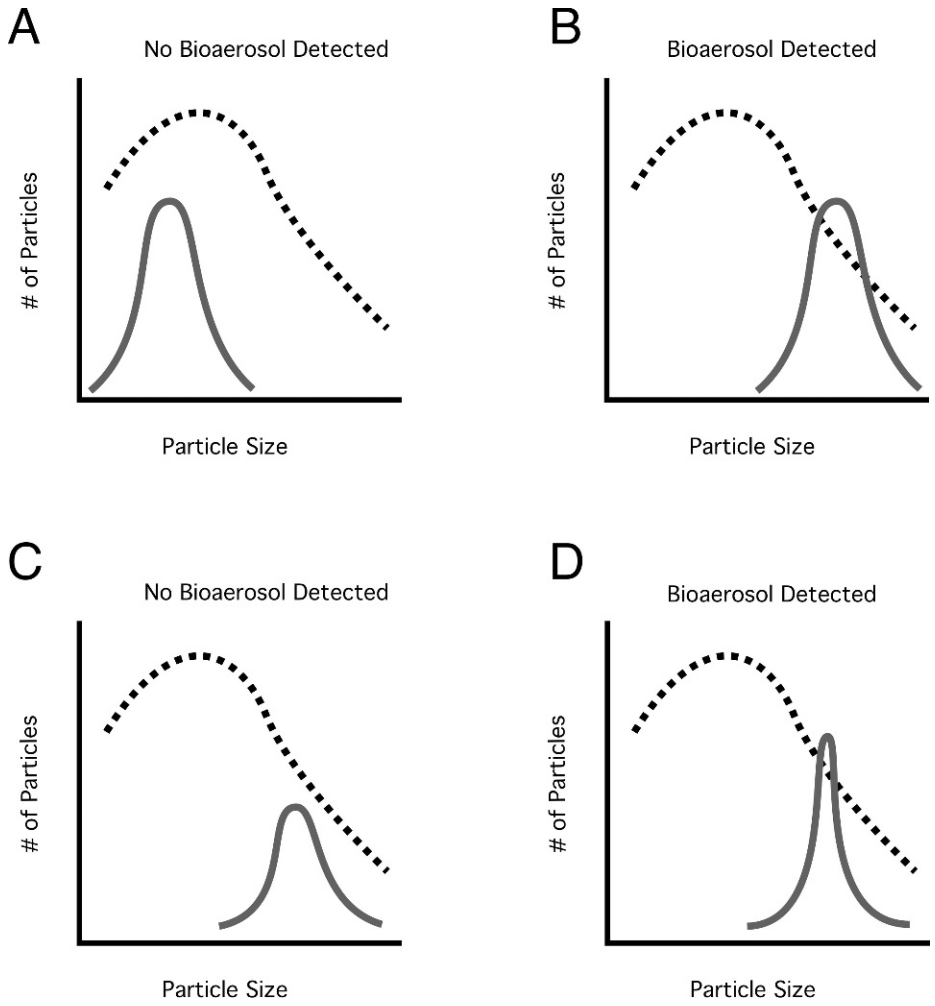

Figure 3. Particle size range and distribution ''shape'' vastly affect detector testing. Standoff detectors have different detection thresholds at different particle sizes (dotted line). Due to this variable sensitivity, different particle size distributions that contain an identical number of particles (solid lines) may or may not be detected (A, B). Beyond the size range, the shape of this distribution can also affect detector performance, as differently shaped distributions that contain an identical range and number of particles may or may not cross the detection threshold (C, D).

tion contained in ACPLA is recorded when measuring and calculating TALA*p*. Therefore, TALA*p* can be co-reported with ACPLA in order to make direct comparisons with legacy ACPLA data, if so desired. For example, 100 TALA (15, 5 microns) would indicate that there are 100 total units of agent per liter of air, distributed amongst 15 ACPLA, with a volume-weighted average particle radius of 5 microns. Granted, PSDs were not specified or standardized in earlier tests, making direct comparisons of TALA*p* with some legacy data potentially problematic. However, even imperfect TALA*p* comparisons will be more useful than their ACPLA-based counterparts, because the differences in total amount of agent and PSD are known and can be incorporated into the comparisons. As mentioned earlier, "identical" ACPLA aerosols used in actual testing have been substantially different (in terms of the amount of agent present), adversely affecting accurate detector evaluations.

The progression from chambers to ambient breeze tunnels to large-scale open air testing introduces additional variability and experimental error. While particle sizing and binning is relatively straightforward in a chamber under well-controlled conditions, outdoor testing introduces a considerable increase in background particulates (i.e., "noise"). The cleanest air in the desert around Dugway Proving Ground had background particulates of ~100 particles per liter (ppl) but can vary up to 5,000 ppl even on a clear day. Some of the background will fall within the 1- to 10-micron size range, emphasizing the need for strict monitoring of the background at all times during the test. In breeze tunnel and field testing, the background particulates may far outnumber those of the bioaerosol

such that simple background subtraction may not be possible. One potential technological fix would be to develop simple taggants for test aerosols that would allow particle sizers to discriminate between bioaerosol and background particles. Something as simple as Green Fluorescent Protein (GFP) could act as a signal to a fluorescently gated particle sizer, indicating that a particular particle should be counted as part of the bioaerosol. GFP is a standard taggant in biological experiments and industrial scale fermentation processes and could readily be used in the environment without restriction. Fluorescently enabled particle sizers are already commercially available. While such a tagging system would need to be tested and verified before wide-scale use, it could substantially increase T&E capabilities.

Measurement of TALA*p*

Knowledge of the PSD is required to determine the TALA, thus TALA*p* is readily generated while determining the TALA. The following calculation assumes a particle counter that provides the number of particles in a given size range (bin) as a function of particle size, taken here as the radius. This type of particle sizer is readily available and already in use in bioaerosol testing facilities. While different particle sizers may operate on different principles (light scattering intensity, aerodynamics, etc.), they all provide the number of particles as a function of size (equivalent radius). If the particles are assumed to be spherical, then the TALA calculation is the same for data from any particle sizer, and the amount of agent in each particle can be calculated in a similar manner. While disseminated biological aerosols (organisms, filler, surfactants, and/or other extraneous materials) are not always strictly spherical, this source of error should fall within an acceptable range for T&E (which is an inherently variable process). While many agents themselves are not spherical at all, their aggregates in the 1- to 10-micron size range aerosol can be. Even when the particles are not spherical, particle sizers generally return data as the radius of an equivalent spherical particle. For the purpose of calculating the volume of a particle, and from there TALA*p*, an assumption of spherical particles is reasonable.

Two parameters from the particle counter are needed: sampling flow rate (F, l/min) and the sampling time (t, min) used to obtain a given data set. Depending upon the instrument, F may or may not be under the experimenter's control but will be known. The time, t, is usually under experimenter control, with larger sampling times leading to more precise PSDs. Depending on the sampling time of the detector itself, as well as the time course of the entire test, it is likely that a number of separate measurements will be taken

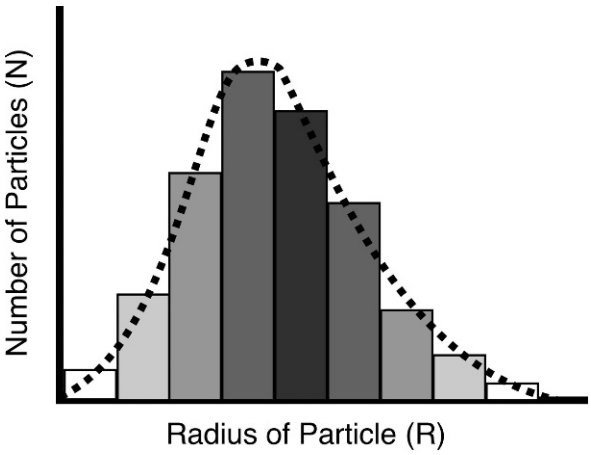

Figure 4. Hypothetical output from a particle size counter. Particle sizers ''count'' the number of particles of various hypothetical radii in various size bins (boxes). The various bins can be fit to a distribution curve (dotted line), if necessary. Particle size data should be corrected for background (see main body of report).

during the course of the run. Clearly, multiple data sets can be suitably averaged, so only a single calculation will be used here. The data are normally collected numerically and are displayed as a histogram as in *Figure 4*. Here, N_i is the number of particles per liter of air in a bin of size delta R centered at R_i.

The actual data from the counter is the number of particles in the size bin N_i' (the number of particles collected by the detector in a given sampling period), which can be converted to N_i by dividing N' by Ft; $N_i = N'/(Ft)$. Since Ft is a constant, one could alternatively use N' in the calculation and then divide by Ft at the end.

Now, the total volume of particles contained in 1 liter of air (V) is given by $4/3\pi \sum N_i R_i^3$. (This sum is derived from the equation for the volume of a sphere: $4/3\pi r^3$). If v is the equivalent volume of one agent in the particle (volume of the agent itself, plus any surrounding filler and/or other material), then

$$TALA = V/v.$$

The parameter v may be obtained from the composition of the disseminated aerosol, plus knowledge of the average volume of the agent itself (V_a). For a dry powder, where w is the weight fraction of the agent in the powder, D is the bulk density of the powder, and D_a is the density of the agent, then $v = (D_a V_a)/(Dw)$. The values of w and D are known from the powder formulation and are under the control of the experimenter or tester. The values of D_a and V_a should be available for each agent (V_a could be calculated via microscopy, for example) and can generally be treated as constants. The values of D_a

are expected to be near 1 g/mL, and it may be that this value can be used for all agents without introducing significant error. If an aqueous slurry is disseminated instead of a dry powder, the value of w to be used in the above expression must be corrected for the weight of water since it will evaporate after dissemination leaving the dry aerosol. The value is corrected by dividing the weight fraction of agent (organisms) in the slurry by 1 minus the weight fraction of water in the slurry. Clearly, this calculation assumes all of the water has evaporated; however, the calculation can be modified to allow some water to remain if test data indicate that the remaining water is a significant source of error. To summarize then, in terms of the experimental (measured) parameters;

$$TALA = (4\pi wD)/(3V_aD_a)\sum N_iR_i^3.$$

If the data from the particle counter can be approximated by an analytical function (e.g., normal, log normal, Poisson) as represented by the dotted line in *Figure 4*, then the summation can be replaced by an integral:

$$TALA = (4\pi wD)/(V_aD_a)\int N(R)R^2dR.$$

A note on the "*p*" in TALA*p*; clearly, the PSD has been measured, and as stated in the body of this report, can be characterized in a number of ways. If a single parameter is desired, perhaps the best one to use is the volume-weighted average radius since it reflects the average size containing the most particles. Finally, it may be noted that with the data collected, it is also possible to weight the distribution for inhalation effectiveness as a function of size if so desired, either now or at some future time.

It should be noted that the equation above allows for one extremely simple way to calculate the total amount of agent in a liter of air. Alternatively, one could use experimental measurements of the amount of agent in a given particle size (i.e., based on electron micrographs of actual particles) rather than relying on w, D_a, and V_a to make a numerical estimate of the amount of agent. In this case, TALA*p* would be calculated by multiplying the number of particles of each size by the number of agents per particle and then summing results, similar to the example in *Figure 2*.

Potential limitations of TALA*p* as a standard unit of measure

We strongly support the proposition that TALA*p* provides a substantial improvement over ACPLA as a standard unit of measure, without requiring the massive investments necessary to implement a more complex unit of measure such as BAULA. However, there are some limitations involved with implementing TALA*p*. As mentioned above, TALA*p* does not contain any information on the amount of live versus dead agent present in the aerosol. While this is not necessarily a concern for the comparative T&E of detectors, it is difficult to translate TALA*p* into a health risk without this information. Thus, without some sort of conversion, health risk will not be captured by a unit such as TALA*p*.

Unfortunately, for agents other than spores, it can be extremely difficult to determine the amount of live agent after it has been dispersed as an aerosol. Simply collecting the agent (via impactors or impingers) can reduce viability since vegetative bacteria and viruses are quite labile and are apt to be destroyed by the collection process itself. Indeed, obtaining accurate measures of viability even in T&E *referee equipment* is a problem that will likely take substantial investments of time and money to properly address.

While a theoretical calculation for health risk would be possible (and a proposed method was presented in the NRC report), in our opinion many of the measures necessary to make this calculation are currently unknown or highly variable, and any final calculation may not be precise enough to accurately represent the health risk. The variables of interest would include breathing rates, amount of live agent, exposure time, agent identity (including subtype), LD_{50} at the given PSD, environmental conditions at time of release, and additional components of the bioaerosol. Some of these variables could be reasonably estimated (i.e., breathing rates), while others will have a large range of possible values (published LD_{50}'s for some agents can vary by orders of magnitude). This topic clearly deserves an in depth study to determine if health hazard can be calculated with any reasonable degree of accuracy.

TALA*p* requires an accurate measurement of the amount of total agent present. This calculation requires knowledge of the volume and density of each agent. Volume measurements are likely available in research literature, though they could be readily obtained (i.e., via microscopy) if past efforts are deemed insufficient. The density of the agent can likely be estimated as 1 g/mL, although more accurate values could be determined through experimental measurements. Thus, it appears that these limitations could be readily overcome with relatively minor research investments.

Finally, TALA*p* relies upon an accurate determination of the number and size of particles. While this count is easily obtained in a well-controlled test chamber, background particles can become problematic in breeze tunnel and field testing. Thus, to obtain an

accurate particle count, it may be necessary to develop testing methodologies that can discriminate between bioaerosol and background particles. Simple tagging methodologies (or other methods), as discussed above, may be able to address this limitation.

In all, TALA*p* has some limitations. It should be noted, however, that these limitations are not specific to TALA*p* and for the most part have plagued other aspects of bioaerosol T&E efforts for years. We believe that the main limitations can be overcome with relatively limited investments, and that these investments will continue to pay dividends to the T&E community beyond the implementation of TALA*p*.

Recommendations

We recommend that a new unit of measure be introduced T&E of both point and standoff aerosol biodetectors. This unit of measure, TALA*p*, contains all of the key components necessary to accurately test and evaluate both bioaerosol point and standoff detectors. Conveniently, TALA*p* applies to all types of agents including spores, vegetative bacteria, and viruses. Thus, TALA*p* can be used as a unit of measure for all agent classes. We recommend that challenge level requirements for biodetectors be stated in TALA*p* with a given PSD (or several different PSDs) and sampling time. TALA*p* is measurable in test chambers with current referee equipment and legacy data should be readily convertible to TALA*p*. In order to accurately measure TALA*p* in breeze tunnels and in the field, additional particle sizing techniques may need to be developed. Thus, we recommend that TALA*p* be implemented along side of ACPLA in the short term for chamber tests and then in breeze tunnels and field tests as protocols allow. In fact, since TALA*p* contains all of the information in ACPLA, TALA*p* can be reported as follows: 100 TALA (15, 5 microns), where there are 100 units of agent per liter of air, distributed amongst 15 agent containing particles (ACPLA), with a volume-weighted average radius (a concise way to describe the PSD) of 5 microns. Such a reporting mechanism would allow immediate comparison of TALA*p* values to existing legacy ACPLA data. Furthermore, this "pilot program" for TALA*p* in test chambers will allow the T&E community time to familiarize themselves with TALA*p* measurements before full implementation. ❑

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Endnotes

[1]The views expressed in this article are those of the authors and do not reflect the official policy or position of the National Defense University, the Department of Defense or the U.S. Government. All information and sources for this paper were drawn from unclassified materials.

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